SERUM FOLATE LEVEL IN CHILDREN WITH PERSISTENT AND CHRONIC DIARRHEA

By

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ABSTRACT

Background: *Chronic diarrhea is a major problem in pediatric patients because it induces malnutrition, general developmental defects, and an extremely death. Thus, it is important to make an accurate diagnosis, find definite etiologies, and apply proper treatments.*

Aim and objectives: *this work designed to study the serum level of folate in children with presistant and chronic diarrhea, Subjects and methods; this case control study was conducted in two groups of children. Group 1: Twenty-five (25) patient suffering from diarrhea for more than fourteen days. Group 2: Twenty-five (25) healthy children of matched age and sex considered as a control group. The duration of the study ranged from 6-12 months, Result; Mean plasma folate levels were significantly lower in cases group (5.01 ±1.26) compared to control group (18.62 ± 5.47), Conclusion; The mean plasma folate levels were significantly lower in diarrheal cases group compared to normal health control group. There was a significant positive correlation between plasma folate with calcium and sodium.*

Keywords: *chronic diarrhea; Serum folate level; pediatric; persistent.*

INTRODUCTION

Diarrhea is defined as excessive loss of fluid and electrolyte in the stool. Acute diarrhea is defined as sudden onset of excessively loose stools of >10 mL/kg/day in infants and >200 g/24 hr in older children **(kliegman et al., 2011)**.

When the duration of diarrhea lasts more than 14 days, it is defined as chronic and persistent diarrhea. Diarrhea is the second leading cause of death in younger children less than 5 years of age in the world **(Wardlaw et al., 2010)**.

It is estimated that diarrheal illnesses are responsible for 2 to 4

million childhood deaths worldwide each year. (In 2002, the World Health Organization estimated that 13.2% of all childhood deaths worldwide were caused by diarrheal diseases, 50% of which were chronic diarrheal illnesses. Large-scale studies indicate that the prevalence of chronic diarrheal illnesses worldwide ranges from 3% to 20%, and the incidence is 3.2 episodes per child- year **(Abba et al., 2009)**.

Most of the serum folate is present in the inactive 5-methyltetrahydrofolat (5-methyl THFA) form.Upon entering cells, 5- methyl THFA demethylates to THFA, the biologically active form involved in folate-dependent enzymatic reactions. It is necessary for the formation of several coenzymes in many metabolic systems, particularly for purine and pyrimidine synthesis, nucleoprotein synthesis and maintenance in erythropoiesis. Folate like vitamin B12 is a provider of 1-carbon residues for DNA and RNA synthesis **(Med Clin North et al., 2017)**.

Poor micronutrient status is a risk factor for several diarrheal illnesses. micronutrient deficiency diminish immune function and this increase the susceptibility of a child to infection, delays full recovery and increase probability of severe illness developing absorbtion of folate, vitamin A, vitamin B12 and minerals like zinc shown to be reduced in diarrhea **(BlackRe et al., 2003)**.

Hereditary folate malabsorption is a disorder that interferes with the body's ability to absorb folates from food. Infants with hereditary folate malabsorption are born with normal amounts of folates in their body because they obtain these vitamins from their mother's blood before birth. They generally begin to show signs and symptoms of the disorder within the first few months of life because their ability to absorb folates from food is impaired **(Kron D et al., 2017)**.

Some infants with hereditary folate malabsorption exhibit neurological problems such as developmental delay and seizures. Over time, untreated individuals may develop intellectual disability and difficulty coordinating **(lasry I et al., 2008)**.

Aims of the Work

The aim of study was to study the serum level of folate in children with presistant and chronic diarrhea.

PATIENTS AND METHODS

This case control study conducted in two groups of children; **Group 1:** Twenty five

(25) patients suffering from diarrhea for more than fourteen days. **Group 2:** Twenty five (25) healthy children of matched age and sex considered as a control group.

Sample size calculation:

The sample size was calculated using Power and Sample size software version 3 (epi info) based on a previous study done by **(Manger et al., 2011)** They reported mean Folate level children with plasma folate concentrations in the lowest quartile had higher odds of persistent diarrhea than children in the other quartiles [adjusted OR = 1.77 (95% CI = 1.14, 2.75); P = 0.01]. This effect differed between boys [adjusted OR = 2.51 (95% CI = 1.47, 4.28)] and girls [adjusted OR = 1.03 (95% CI = 0.53, 2.01); P

Sample size:

The sample size will be

$$n = 2\left[\frac{(Z_{\alpha/2} + Z_{\beta}) * \sigma}{\mu_1 - \mu_2}\right]^2$$

calculated using the following formula:

Where:

n = sample size

Zα/2 = 1.96 (The critical value that divides the central 95% of the Z distribution from the 5% in the tail)

Zβ = 0.84 (The critical value that separates the lower 20% of the Z distribution from the upper 80%)

σ = the estimate of the standard deviation of the children with plasma folate concentrations in the lowest quartile = 1.77

μ1 = mean children with plasma folate concentrations in the lowest quartile was = 26.9

μ2 = mean in children with plasma folate concentrations in the other quartiles fpllow up = 8.9

So, by calculation, the sample size will be equal to 50 patients in total.

Inclusion criteria: Age: 6months to 6 years, children with diarrhea for more than 14 days and no folate suplmentation in the last six monthes.

Exclusion criteria: Child with diarrhea less than 14 days, children with folic acid supplementation, children known to have folate difficiency, children with current disease (severe malnutrition, encephalitis, meningities, sepsis and bronchopneumonia), patients receive trimethoprim and sulphonamide and patients receive large dose of vitamin A in the last two monthes.

These children subjected to a full history, full clinical examination and anthropometric measurements, laboratory investigations.

Clinical Study: This included

I. Complete history taking.

II. Physical examination: using the pediatric clinic sheet and the anthropometric measurements taken at clinic as follows: Body Weight (in kg), standing Height (in cm) and body mass index (BMI).

III. Laboratory Investigations including: serum folate levels by (ELISA) using red-ELISA ket catalogue NO 201-12-1510 China by New-Test company.

Complete blood count using (Auto hematology analyser), renal and liver functions if indicated, stool analysis and culture if indicated, venous blood gases and serum electrolytes (Na, K and CA) by flame photometry.

Ethical considerations:

1. An informed consent taken from all parents before getting involved in study. Confidentiality of all data ensured.
2. The parents have the right to withdraw from the study at any time without giving any reasons.
3. The study was done after approval of ethical committeesof Pediatrics department & faculty of medicine for Al-Azhar University.

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Data management and Statistical Analysis: Data collected throughout history, basic clinical examination, laboratory investigations and outcome measures coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance, correlation by Pearson's correlation or Spearman's. P value was set at <0.05 for significant results & <0.001 for high significant result.

RESULTS

Table (1): Demographic data of the two studied groups

Variable		**Cases** (n=25)	**Controls** (n=25)	**t / χ^2**	**P**
Age (years) Mean ± SD		4.11 ± 2.83	3.52 ± 2.76	.746	.459
Sex	Female	11 (44%)	12 (48%)	.081	.777
	Male	14 (56%)	13 (52%)		
Mother education					
Illiterate		2(8%)	1(4%)	1.07	.873
Iducated		23(92%)	(24)96%		
Residence					
Rural		4(16%)	5(25%)	.4	.818
Urban		21(84%)	20(80%)		

There is no significant difference between the two studied groups regarding demographic data **Table (1)**.

Table (2): Clinical presentation distribution among the cases group

	Cases (n=25)
Fatigue	3 (12%)
GIT problems	8 (32%)
Irritability	3 (12%)
Pale	2 (8%)
Anorexia	5 (20%)

The most common presentation was GIT problems (32%) and anorexia (20%)

Table (3): Laboratory finding in the two studied groups

	Cases (n=25)	**Controls** (n=25)	**t**	**P**	**Normal levels**
Plasma folate (nmol/L) Mean ± SD	5.01 ± 1.26	16.82 ± 4.57	**12**	**<0.001**	11.3-47.6
Calcium (mg/dl) Mean ±SD	7.6 ± 1.43	8.18 ±1.05	**1.52**	**.135**	9-11
Sodium (mmol/l) Mean ±SD	131.18 ± 9.54	136.28 ± 6.33	**2.23**	**.031**	135-145

Mean plasma folate levels were significantly lower in cases group (5.01 ±1.26) compared to control group (18.62 ± 5.47) **Table (3)**.

Table (4): Nutritional status distribution among the cases group

		Cases (n=25)
Stunting	Positive	5 (20%)
	Negative	20 (80%)
Underweight	Positive	2 (8%)
	Negative	23 (92%)
Wasting	Positive	1 (4%)
	Negative	24 (96%)

Stunting was found in 20% of the patients while underweight found in 2 patients, (8%) and wasting was found in only one patient **Table (4)**.

Table (5): Correlation between plasma folate with other parameters in cases group

Cases	Plasma folate	
	r	P
Age	.111	.444
Weight	.201	.157
Height	.189	.235
Hb	.104	.474
TLC	.114	.429
PLT	.137	.342
RBS	.168	.242
Serum Creatinine	.136	.245
BUN	.116	.120
AST	-.191	.154
ALT	-.186	.103
Calcium	**.376**	**.027**
Potassium	-.246	.081
Sodium	**.357**	**.021**
PH	.282	.165

There is a significant positive correlation between plasma folate with calcium and sodium **Table (5)**.

DISCUSSION

Poor micronutrient status is a risk factor for severe diarrheal illness. Although zinc and vitamin A have received the most attention, folate has also been implicated. Tetrahydrofolate are required for DNA and RNA synthesis. Because of their intertwined metabolic pathways, a poor supply of either folate or cobalamin can lead to functional intracellular folate deficiency.

The main aim of this study was to study the serum level of folate in children with persistent and chronic diarrhea.

There was no significant difference between the two studied groups regarding age, sex, weight & height. The mean age of cases was 4.11 ± 2.83 years. More than half of them (56%) were males.

Our results were in agreement with study of (Khan et al., 2008)

As they reported that there was no significant difference between diarrheal and control groups as regard age and sex.

While, in the study of **(Manger et al., 2011)** the age of the children was 15.3 ± 7.5 mo and 52.5% were boys.

Moreover, **(Ulak et al., 2014)** demonstrated that a total of 823 children were included for cobalamin and folate biomarker analyses; the mean age was 15.7 months and 465 (56%) were boys. The prevalence of stunting, underweight, and wasting (<-2 Z score of length or height for age, weight for age and weight for length or height) were 34%, 25% and 10% respectively.

The present study showed that as regard clinical presentation distribution among the cases group; the most common presentation was GIT problems (20%).

In accordance with our results, study of **(Khan et al., 2008)** as they reported that watery diarrhea and vomiting was the most common clinical presentation among their cases.

Folate deficiency occurs when there is not enough folate present in the body. Some symptoms of folate deficiency include: fatigue, trouble concentrating, headache, irritability, heart palpitations, GIT problems, change in color of the skin, hair, or fingernails & irritability, headache, heart palpitations, and shortness of breath.

Poor micronutrient status is a risk factor for several diarrheal illnesses. Micronutrient deficiency diminishes immune function and this increase the susceptibility of a child to infection, delays full recovery and increase probability of severe illness developing absorption of folate, vitamin A, vitamin B12 and minerals like zinc shown to be reduced in diarrhea **(Thiagarajah et al., 2020)**.

In the study in our hands, mean plasma folate levels were significantly lower in cases group (5.01 ±1.26) compared to control group (18.62 ± 5.47).

There was a significant positive correlation between plasma folate with calcium and sodium.

Our results were supported by study of **(Manger et al., 2009)** as they reported that children with plasma folate concentrations in the lowest quartile had higher odds of persistent diarrhea than children in the other quartiles [adjusted OR = 1.77 (95% CI = 1.14, 2.75); P = 0.01].

Also, **(Rizwan & Gupta et al., 2012)** revealed that persistent diarrhea was significantly associated with low folate level (adjusted OR 1.77; 95% CI 1.14–2.75; p=0.010).

The observed association between poor folate status and persistent diarrhea is consistent with the notion that micronutrient

status is associated with the severity, rather than the incidence, of diarrheal illness **(Fisher et al., 2007)**.

Although persistent diarrhea accounts for only a small proportion of all diarrhea episodes, it accounts, together with dysentery, for 65% of all diarrhea-associated deaths in India **(Bhandari et al., 1992)**.

There are few studies reporting on the association between folate or cobalamin and diarrhea in children. In a RCT among 6- to 23-mo-old Bangladeshi children with acute diarrhea, there were no differences in diarrheal duration or stool output between children receiving folic acid (5 mg at 8-h intervals for 5 d) or placebo **(Ashraf et al., 1998)**.

Provision of a snack to 5- to 12-y-old Columbian school children was associated with increased plasma cobalamin concentrations and fewer reported days with morbidity symptoms including diarrhea. However, the intervention was not randomized and the effect on morbidity could also be related to increased energy intake or other nutrients in the snack **(Arsenault et al., 2009)**.

Only measure of recent illness was mothers' reports of diarrhea 24h prior to inclusion in the study. However, the proportions with self-reported diarrhea in the past 24 h before inclusion were similar among children in the low- and reference folate groups and inclusion of this variable in multivariate analyses did not change their results. In addition, the most important predictor of folate status was whether a child received breast milk, an important source of folate for young children. Therefore, it is likely that the folate deficiency associated with persistent diarrhea in this study was related to dietary intake. Should, however, infection be the cause of poor folate status, folic acid supplementation may be necessary to break the cycle of defective folate uptake and infections **(Mackey et al., 2009)**.

In the study of **(Ulak et al., 2010)** the low prevalence of folate deficiency may be due to the high proportion of breastfed children. The folate concentration is high in breast milk which is, to a large extent, independent of folate status of the mother. It may also be due to frequent consumption of foods like cereals, legumes and green leafy vegetables, which are rich sources of folate.

CONCLUSION

The mean plasma folate levels were significantly lower in diarrheal cases group compared to normal health control group.

There was a significant positive correlation between plasma folate with calcium and sodium.

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الملخص العربي

يُعـرَّف الإسـهال بأنـه فقـدان مفـرط للسـوائل والكهـارل فـي البـراز. يُعـرَّف الإسـهال الحـاد بأنـه ظهـور مفـاجئ للبـراز الرخـو المفـرط بمقـدار> 10 مـل / كجـم / يـوم عنـد الرضـع و> 200 جـم / 24 سـاعة عنـد الأطفـال الأكبـر سـنًا. عنـدما تسـتمر مـدة الإسـهال أكثـر مـن 14 يومًـا، يـتم تعريفهـا علـى أنهـا إسـهال مـزمن ومسـتمر. الإسـهال هـو السـبب الرئيسـي الثـاني للوفـاة بـين الأطفـال الأصـغر سنًا الذين تقل أعمارهم عن 5 سنوات في العالم.

يعتبـر الإسـهال المـزمن مشـكلة رئيسـية لـدى مرضـى الأطفـال لأنـه يتسـبب فـي سـوء التغذيـة وعيـوب النمـو العامـة والوفـاة الشـديدة. وبالتـالي، مـن المهـم إجـراء تشـخيص دقيـق وإيجاد مسببات محددة وتطبيق العلاجات المناسبة.

يعـاني الأطفـال المصـابون بسـوء امتصـاص حمـض الفوليـك الـوراثي مـن صـعوبات فـي التغذيـة وإسـهال وفشـل فـي زيادة الوزن والنمو بالمعدل المتوقع (فشل النمو).

سيصـمم هـذا العمـل لدراسـة مسـتوى حمـض الفوليـك فـي الدم لدى الأطفال المصابين بالإسهال المزمن والمقاوم.

أجريـت دراسـة الحالـة هـذه فـي مجمـوعتين مـن الأطفـال. المجموعـة الأولـى: خمسـة وعشـرون (25) مـريض يعـانون مـن الإسـهال لأكثـر مـن أربعـة عشـر يومًـا. المجموعـة الثانيـة: خمسـة

وعشرون (25) طفلًا يتمتعون بصحة جيدة من نفس العمر والجنس يعتبرون مجموعة ضابطة. تراوحت مدة الدراسة من 6 إلى 12 شهرًا.

أظهرت النتائج الرئيسية للدراسة ما يلي:

لا يوجد فرق معنوي بين المجموعتين المدروستين فيما يتعلق بالبيانات الديموغرافية.

أكثر الأعراض شيوعاً كان الصداع (32٪) ومشاكل الجهاز الهضمي (20٪).

لا يوجد فرق معنوي بين المجموعتين المدروستين فيما يتعلق بالمعايير المختبرية.

هناك فرق معنوي بين المجموعتين المدروستين فيما يتعلق بالصوديوم.

كان متوسط مستويات الفولات في البلازما أقل بشكل ملحوظ في مجموعة الحالات (5.01 ± 1.26) مقارنة بمجموعة التحكم (18.62 ± 5.47).

توجد علاقة ارتباط موجبة معنوية بين حمض الفوليك في البلازما والكالسيوم والصوديوم.